GABA and high activity of GAD in the β-cell tumor, this result suggests that GABA is synthesized and localized within β-cells in the islets and probably does not originate in the nerve terminals as in the CNS. In the islets, GABA may be involved in functions such as the synthesis and release of insulin, the synthesis of protein, the supply of energy through the GABA shunt, or neurotransmission as in the CNS. Recently, other pathways of GABA synthesis have been reported, from putrescine (*18*) and glutamate (*19*). Although we found high GAD activity in the islets of Langerhans in parallel with the presence of high amounts of GABA, part of the GABA found in the islets may be derived through these other pathways. Further studies of the function and distribution of GABA in the pancreatic islets might clarify the role of GABA in the mammalian CNS.

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11 May 1976; revised 8 July 1976

Amianthoid Change: Orientation of Normal Collagen Fibrils During Aging

Abstract. *High-angle x-ray diffraction provides direct evidence that amianthoid change, occurring during aging of costal cartilage, corresponds to a transformation from an isotropic to a marked anisotropic distribution of collagen fibrils. Low-angle x-ray diffraction and electron microscopy show that the fibrils have the customary 67-nanometer axial periodicity. Electron microscopy shows that wide amianthoid collagen fibrils consist of smaller parallel fibrils fused together. Similarities between amianthoid change and tendon morphogenesis are briefly discussed. Amianthoid change is remarkable in that aging is accompanied by increased order.*

We have used x-ray diffraction and electron microscopy to investigate the amianthoid changes that occur during the aging of costal (rib) cartilage. Amianthoid areas are seen as opaque white flecks in the surrounding yellow, translucent hyaline cartilage (*1*); under the light microscope they have a fibrous appearance, which has led to the terms "fibrillation," "fibrillary transformation," and "fibrous transformation" for the changes (*2*). It has been postulated that age-dependent changes may be the first stage in the development of osteoarthrosis (osteoarthritis) which occurs when the articular cartilage lining synovial joints is eroded (*3*).

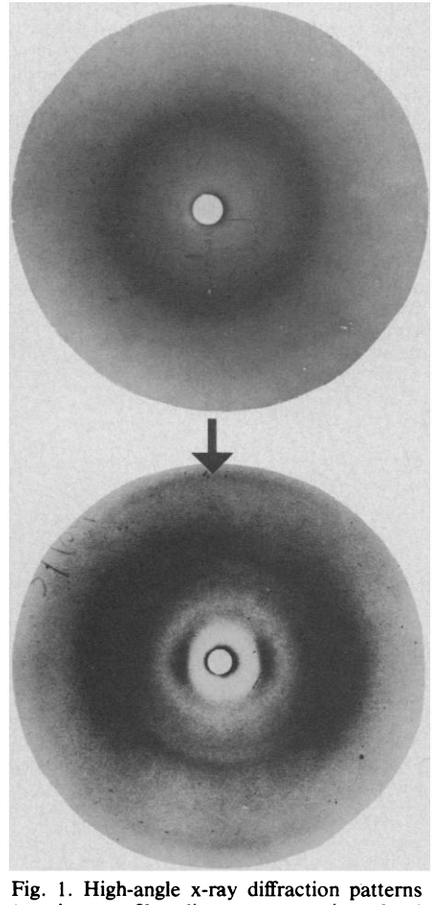

Fig. 1. High-angle x-ray diffraction patterns (specimen-to-film distance approximately 4 cm) from cartilage in transverse section of rib. (Top) There is no sign of orientation in the organization of the fibrils in the cartilage. (Bottom) The pattern from an amianthoid area is diagnostic of oriented collagen; the arrow points to the characteristic 0.29-nm meridional reflection.

Our high-angle x-ray diffraction patterns, obtained from the cartilage in transverse sections of human rib (*4*), provide new and direct evidence that amianthoid areas consist of oriented collagen fibrils. Although patterns from normal areas (Fig. 1, top) show no detectable orientation in any of their components, the patterns (Fig. 1, bottom) from amianthoid areas are clearly characteristic—in general, in intensity distribution, and, in particular, in the 0.29-nm meridional reflection—of partially oriented collagen fibrils (*5*). According to criteria listed by Ramachandran (*6*) this result provides direct evidence (so far as we know, the first) for amianthoid areas consisting of oriented collagen fibrils; additional direct evidence comes from our low-angle x-ray diffraction patterns and electron micrographs. Previously, the only available evidence for amianthoid areas consisting of collagen was from enzymatic studies and electron microscopy (*2*).

Low-angle x-ray diffraction patterns of amianthoid areas show that their oriented collagen fibrils have an axial periodicity which is not significantly different from that expected of normal fibrils. Measurements of electron micrographs of thin sections of fixed and stained amianthoid areas had previously suggested that their fibril periodicities were shorter than usual at between 56 and 62 nm (*2*). It is notoriously difficult to obtain accurate estimates of periodicity from micrographs of sectioned material in which, for example, fibrils may not have been cut exactly longitudinally. Further, the material has to be subjected to considerable chemical treatment, including exhaustive dehydration. X-ray diffraction measurements do not suffer from such drawbacks. Figure 2 shows that our patterns yield a value of 67 ± 1 nm for the periodicity of amianthoid collagen fibrils (*7*). This value is clearly comparable to values obtained for tendon collagen (*8*, *9*). Unfortunately, no com-

parable values are yet available for normal cartilage collagen; recording low-angle x-ray diffraction patterns from the low concentration of disoriented fibrils is difficult, but electron micrographs of fibrils from disintegrated cartilage, tendon, and other collagens yield similar values after fixation, staining, and drying (*10*). We conclude that the periodicities, and presumably the axially projected structures (*11*), of amianthoid and normal cartilage collagen fibrils are not significantly different.

We have obtained electron micrographs from homogenized (*12*) amianthoid areas which confirm that their collagen fibrils have normal periodicities but relatively large diameters; the fibrils appear to consist of smaller, parallel fibrils fused in register. Micrographs of sectioned material suggested that amianthoid areas contain tactoids of exceptionally large diameters (up to 10^3 nm) (*2*) as compared with the smaller diameters (35 to 120 nm) (*13*) of fibrils from normal hyaline cartilage. Although our observations of homogenates confirm that some very large tactoids are present, they were so heavily stained that we have only been able to obtain micrographs of the smaller ones (diameters, 50 to 190 nm). Figure 3 shows that these tactoids consist of smaller fibrils fused together. Individual constituent fibrils have an axial banding pattern similar to that of other collagens. Fibrils appear to be fused parallel with their bands in register throughout the width of the tactoid. The axial periodicity (66 ± 2 nm) of the fibrils was unexceptional. We conclude, once again, that amianthoid and normal collagen fibrils have the same axially projected structures, but that amianthoid areas contain tactoids consisting of parallel bundles of fibrils.

X-ray diffraction has proved to have several advantages for determining the orientation of collagen fibrils in cartilage; not the least is its ability to yield information from a thick (1 mm) section of material. Electron microscopy has been extensively used for this purpose, but comparable results could be obtained only with an accurate and efficient method of scanning micrographs (*14*), and only then by serial sectioning or very delicate microdissection of material. However, we are aware of only three previously published x-ray diffraction studies of cartilage (*15*).

The arrangement of collagen fibrils in amianthoid areas of cartilage and in tendon are similar in two respects. First, tendon and amianthoid collagen fibrils both show a considerable degree of orientation. Second, in tendon the fibrils are

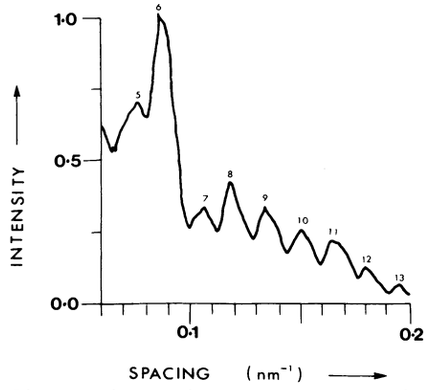

Fig. 2. Microdensitometer trace along the meridional direction of a low-angle x-ray diffraction pattern (the distance from specimen to film is approximately 10 cm) from an amianthoid area. Peaks correspond to diffraction orders (marked 5 to 13) from a periodic spacing of 67 ± 1 nm; peaks of order < 5 could not be recorded at this distance of specimen to film. The strongest recorded peak height is arbitrarily given a value of 1.

arranged both parallel and antiparallel with their so-called a-bands in register (*16*); in amianthoid the parallel, but not apparently the antiparallel, arrangement exists.

Aging of cartilage is accompanied by depletion of glycosaminoglycans surrounding the collagen fibrils (*17*), and amianthoid areas are particularly deficient (*2*), so that the concentration of glycosaminoglycan becomes closer to the low value found in tendon. The glycosaminoglycan-containing proteoglycans are more easily leached out of osteoarthrotic as compared to normal articular cartilage (*18*); this implies that similar polysaccharide depletion may be involved in osteoarthrosis. Another consideration is that chemical composition, as well as the quantity, of glycosaminoglycan in extracellular matrices changes with age (*19*).

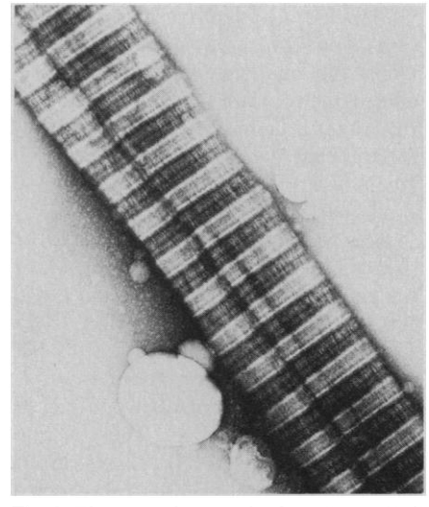

Fig. 3. Electron micrograph of a collagen fibril from an amianthoid area, negatively stained with ammonium molybdate. On close inspection this micrograph shows several fibrils fused together with their alternating light and dark stained bands in register. The micrograph was originally taken at a magnification of 62,500; in this figure the scale is given by the axial periodicity at 66 ± 2 nm measured from this and a series of similar micrographs. The left-hand "fibril" is only approximately in register.

We cannot yet distinguish whether depletion of glycosaminoglycan leads to orientation and fusion of collagen fibrils, whether orientation and fusion lead to exclusion of proteoglycan, or whether both effects have a further common cause. Distinguishing between the three hypotheses would further our understanding of tendon morphogenesis and of aging in cartilage. The possible role of increased cross-linking in amianthoid change has been discussed (*2*). It is remarkable that aging of costal cartilage is associated with an increase in order in the arrangement of its collagen fibrils; increasing order often occurs during development of biological systems, but aging changes are usually associated with increasing disorder.

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24 May 1976

β-Adrenergic Receptor Involvement in 6-Hydroxydopamine-Induced Supersensitivity in Rat Cerebral Cortex

Abstract. *The intraventricular administration of 6-hydroxydopamine, a procedure which destroys noradrenergic nerve terminals in the central nervous system, caused an increase in the density of β-adrenergic receptors in rat cerebral cortex, without affecting their affinity for isoproterenol. The results suggest that changes in the density of adrenergic receptors are involved in 6-hydroxydopamine-induced supersensitivity at central noradrenergic synapses.*

Interaction of the neurotransmitter norepinephrine (NE) with β-adrenergic receptors leads to activation of the enzyme adenylate cyclase (E.C. 4.6.1.1) and to an increase in the intracellular production of adenosine 3′,5′-monophosphate (cyclic AMP) (*1*). Denervation of peripheral organs results in an enhanced response to exogenous NE involving both pre- and postsynaptic components (*2*). The former component reflects the loss of the presynaptic nerve terminals and the associated NE uptake system. Injection of 6-hydroxydopamine (6-OHDA) into the lateral ventricle causes a specific degeneration of central catecholaminergic nerve terminals and a depletion of catecholamines (*3, 4*). Noradrenergic neurons innervate the cerebral cortex and play an important role in regulating many behavioral states. It is therefore important to study the effect of denervation on the properties of these synapses.

In slices of rat cerebral cortex, activation of either α- or β-adrenergic receptors leads to an increased production of cyclic AMP (*5, 6*). Administration of 6-OHDA increased the production of cyclic AMP in response to activation of both types of receptors (*7, 8*). To characterize the molecular basis for this increase in responsiveness, several components of the β-adrenergic receptor–adenylate cyclase system were studied in rats that had been treated with 6-OHDA. Isoproterenol-stimulated accumulation of cyclic AMP was determined in slices of rat cerebral cortex. In the same experiments, the properties and density of β-adrenergic receptors were determined by using a potent β-adrenergic receptor antagonist, ^{125}I-labeled hydroxybenzylpindolol (HYP) (*9, 10*), as a radioactive ligand. Only the response mediated by β-adrenergic receptors was investigated in this study. Isoproterenol is a specific β-adrenergic receptor agonist, and ^{125}I-labeled HYP binds specifically to β-adrenergic receptors (*9, 10*).

Male Sprague-Dawley rats (120 to 160 g) were injected intraventricularly (*3, 11*) on each of two successive days with 200 μg (free base) of 6-OHDA (Regis Chemical) dissolved in 20 μl of 0.9 percent saline containing sodium ascorbate (1 mg/ml, *p*H 5). Controls were injected with 20 μl of vehicle. This dose of 6-OHDA decreased NE levels in the cerebral cortex by 81 ± 3 percent (N = 21). Rats were killed by decapitation 7 to 9 days after the first injection. The entire cerebral cortex from each rat was dissected free of midbrain structures, sliced (1 by 0.26 by 0.26 mm) with a McIlwain tissue chopper and resuspended in 15 ml of oxygenated (95 percent O_2 and 5 percent CO_2) Krebs-Ringer buffer (*5, 12*).

Approximately 20 percent of each resuspended cortex was homogenized in 15 ml of 0.32M sucrose, 10 mM tris, *p*H 7.5, by means of a motor-driven Teflon-glass homogenizer. These homogenates were centrifuged at 20,000g for 10 minutes. The resulting pellets were resuspended (200 ml per gram of original wet weight) in 0.9 percent NaCl, 20 mM tris, *p*H 7.5, for use in binding studies which were performed as previously described (*10*). The remainder of the chopped tissue was used to measure isoproterenol-induced cyclic AMP accumulation. A modification (*5*) of the method of Shimizu *et al.* (*12*) was utilized to follow the conversion of tritiated adenosine triphosphate (ATP) to tritiated cyclic AMP. For these experiments the tissue ATP pools were first labeled by incubating the slices for 30 minutes with tritiated adenine (2.5 μc/ml of resuspended tissue). The concentration-dependent effects of isoproterenol on the conversion of tritiated ATP to tritiated cyclic AMP were then followed. Isobutylmethylxanthine (1 mM), an inhibitor of phosphodiesterase (E.C. 3.1.4.1), was included in these experiments. Results are expressed as the percentage conversion of tritiated ATP to tritiated cyclic AMP (*12*). Concentrations of NE (*13*) and protein (*14*) were determined as previously described.

The accumulation of cyclic AMP in response to a maximally stimulating concentration of isoproterenol was 80 ± 5 percent (N = 13) greater in the 6-OHDA treated rats than in the controls (Fig. 1). Treatment of animals with 6-OHDA did not affect the concentration of *l*-isoproterenol needed to produce a half-maximal increase (ED_{50}) in the accumulation of cyclic AMP in slices of cerebral cortex (Fig. 1, inset). The ED_{50} determined from the data presented in Fig. 1 together with the data from two similar experiments was 33 ± 1 nM (N = 13) for controls and 34 ± 2 nM (N = 14) for treated rats.

The binding of ^{125}I-labeled HYP to a particulate fraction derived from rat cerebral cortex has properties similar to those which would be expected of binding to β-adrenergic receptors in vitro. The binding is reversible, saturable, of high affinity, stereospecific, and it is inhibited by appropriate β-adrenergic receptor ligands (*10*). Specific binding (approximately 80 percent of the total ^{125}I-labeled HYP binding) was defined as binding which was inhibited by 0.3 μM *dl*-propranolol. The density of β-adrenergic receptors in the cerebral cortex of 6-OHDA treated and control rats was determined by measuring the specific binding of various concentrations of ^{125}I-labeled HYP and analyzing the data by the